

**CELL DIVISION IN *BACILLUS SUBTILIS*:  
NEW INSIGHTS FROM AN OLD  
MUTANT**

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A thesis submitted in fulfilment of the  
requirements for the degree of  
Doctor of Philosophy



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## **Certificate of Authorship/Originality**

I certify that the work presented in this thesis has not previously been submitted for a degree, nor has it been submitted as part of the requirements for a degree except as fully acknowledged within the text. I also certify that the written preparation of the thesis, and all experimental work associated with it, has been carried out solely by me, unless otherwise indicated. Finally, I certify that all information sources and literature used are acknowledged in the text.

A handwritten signature in black ink, appearing to read 'L Monahan', written in a cursive style.

Leigh Monahan

April 2008

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## Abbreviations

A <sub>(x)</sub>	absorbance (where x = wavelength in nanometres)
Ap <sup>R</sup>	ampicillin resistance marker
AR	analytical reagent
ATP	adenosine 5'-triphosphate
<i>B.</i>	<i>Bacillus</i>
BP	band pass
bp	base pair(s)
BSA	bovine serum albumin
<i>C.</i>	<i>Caulobacter</i>
CCD	charge-coupled device
CFU	colony forming unit
Cm <sup>R</sup>	chloramphenicol resistance marker
Da	dalton(s)
DAPI	4'6-diamidino-2-phenylindole
dATP	deoxyadenosine 5'-triphosphate
dCTP	deoxycytosine 5'-triphosphate
DEAE	diethylaminoethyl
dGTP	deoxyguanosine 5'-triphosphate
DNA	deoxyribonucleic acid
dTTP	deoxythymidine 5'-triphosphate
<i>E.</i>	<i>Escherichia</i>
ECL	enhanced chemi-luminescence
ECT	electron cryotomography
EDTA	ethylenediaminetetraacetic acid
EGTA	ethyleneglycol-bis(2-aminoethyl ether)-tetraacetic acid
Em <sup>R</sup>	erythromycin resistance marker
FRAP	fluorescence recovery after photobleaching
<i>fts</i>	filamentous temperature sensitive
g	centrifugal force (× unit gravitational field)
g	gram(s)

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GDP	guanosine 5'-diphosphate
GFP	green fluorescent protein
GTP	guanosine 5'-triphosphate
h	hour(s)
HPUra	6-( <i>p</i> -hydroxyphenylazo)-uracil
IFM	immunofluorescence microscopy
IPTG	isopropyl-1-thio- $\beta$ -D-galactopyranoside
k	kilo- ( $10^3$ )
kb	kilobase(s)
LP	long pass
m	milli- ( $10^{-3}$ )
<i>M.</i>	<i>Methanococcus</i>
M	moles per litre
MCS	multiple cloning site
min	minute(s)
mol	mole(s)
MOPS	3-( <i>N</i> -morpholino)propanesulfonic acid
MQW	Milli-Q <sup>®</sup> purified water (Millipore)
mRNA	messenger ribonucleic acid
N	any nucleotide
n	nano- ( $10^{-9}$ )
NA	not applicable
p	pico- ( $10^{-12}$ )
<i>P</i>	probability
<i>P.</i>	<i>Prostheobacter</i>
PBS	phosphate-buffered saline
PCR	polymerase chain reaction
$P_{spac}$	IPTG-inducible promoter
$P_{xyl}$	xylose-inducible promoter
RNA	ribonucleic acid
ROW	reverse osmosis purified water
<i>S.</i>	<i>Streptomyces</i>

SDS	sodium dodecyl sulfate
SDS-PAGE	sodium dodecyl sulfate-polyacrylamide gel electrophoresis
SEM	standard error of the mean
TBAB	tryptose blood agar base
TBS	tris-buffered saline
TEMED	N,N,N',N'-tetramethyl-ethylenediamine
Tris	tris(hydroxymethyl)methylamine
U	units (enzyme activity)
UV	ultraviolet
v/v	volume per volume
w/v	weight per volume
YFP	yellow fluorescent protein
μ	micro- ( $10^{-6}$ )

## Publications

### Journal article

Michie, K. A., **Monahan, L. G.**, Beech, P. L., and Harry, E. J. (2006) Trapping of a spiral-like intermediate of the bacterial cytokinetic protein FtsZ. *Journal of Bacteriology* **188**: 1680-1690.

### Book chapter

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### Conference proceedings

Peters, P. C., **Monahan, L. G.**, and Harry, E. J. (March, 2008) Targeting cell division: The Z ring assembles from a dynamic helical-like structure. **SEMINAR**, *Gordon Research Conference on Antibacterial Discovery and Development*, Il Ciocco, Italy.

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**Monahan, L. G.**, and Harry, E. J. (June, 2007) Cell division in *Bacillus subtilis*: Insights into Z ring assembly from a novel mutant. **POSTER**, *Functional Genomics of Gram-Positive Microorganisms, the 14<sup>th</sup> International Conference on Bacilli*, Tirrenia, Italy.

**Monahan, L. G.**, and Harry, E. J. (September, 2006) Bacterial cell division: Insights into Z ring assembly and its regulation from a novel mutant. **POSTER**, *ComBio 2006, the 50<sup>th</sup> Annual Meeting of the Australian Society for Biochemistry and Molecular Biology*, Brisbane, Australia.



**Monahan, L. G.**, and Harry, E. J. (June, 2006) Bacterial cell division: Trapping a helical intermediate in the assembly of the Z ring. **SEMINAR**, 48<sup>th</sup> Annual Meeting of the Australian Society for Microbiology, Gold Coast, Australia.

**Monahan, L. G.**, Michie, K. A., Beech, P. B., and Harry, E. J. (September, 2005) Trapping a spiral-like intermediate of the bacterial cytokinetic protein FtsZ. **SEMINAR**, ComBio 2005, the 49<sup>th</sup> Annual Meeting of the Australian Society for Biochemistry and Molecular Biology, Adelaide, Australia.

Harry, E. J., **Monahan, L. G.**, and Michie, K. A. (July, 2005) Rings and spirals of the cell division protein FtsZ: New lessons from an old mutant. **POSTER**, 2<sup>nd</sup> American Society for Microbiology Conference on Prokaryotic Development, Vancouver, Canada.

Michie, K. A., **Monahan, L. G.**, Beech, P. B., and Harry, E. J. (May, 2005) The earliest step in bacterial cell division: New lessons from an old mutant. **SEMINAR**, 8<sup>th</sup> Australian Bacterial Pathogenesis Meeting, Couran Cover Island, Australia.

Michie, K. A., **Monahan, L. G.**, and Harry, E. J. (September, 2004) Insights into FtsZ from a temperature sensitive mutant of *Bacillus subtilis*. **POSTER**, 46<sup>th</sup> Annual Meeting of the Australian Society for Microbiology, Sydney, Australia.

## Abstract

In bacteria, cell division is mediated by a macromolecular complex consisting of numerous proteins that act together to split the cell into two. The earliest event in this process is the formation of a polymeric ring, composed of the tubulin-like protein FtsZ, at the future site of division. This so-called ‘Z ring’ plays a pivotal role in the cell division mechanism, at least in part because it serves as a scaffold for the assembly of the division apparatus. Importantly, Z ring formation establishes both when and where the cell will divide, and is therefore subject to stringent spatiotemporal control. This thesis is concerned with the molecular mechanism of Z ring assembly and its regulation in the gram-positive model organism *Bacillus subtilis*. It involves the use of powerful fluorescence microscopy techniques in combination with molecular biological and genetic methods to examine the specific effects of a temperature sensitive FtsZ mutation on Z ring formation *in vivo*. The *B. subtilis* strain harbouring this mutation is known as *tsI*, while the mutant protein itself has been designated FtsZ(Ts1).

Initial experiments examined the intracellular localisation pattern of the FtsZ(Ts1) protein in live cells growing at 49°C (the non-permissive temperature for *tsI*). This work revealed that while FtsZ(Ts1) is unable to form Z rings under non-permissive conditions, it retains the capacity to polymerise *in vivo* and instead assembles into short helical-like structures. Interestingly, these helices were observed to reorganise into fully functional Z rings following a shift from 49°C down to permissive temperatures. These and other observations suggest an exciting new model for Z ring assembly in wild-type bacterial cells, involving a regulated helix-to-ring remodelling of FtsZ polymers. The work also suggests that at non-permissive temperatures, the FtsZ(Ts1) protein is unable to complete the final stages of this remodelling process, and becomes trapped as a short helical intermediate of Z ring formation *in vivo*.

To explore how the FtsZ helix-to-ring assembly mechanism is orchestrated within the cell, further experiments aimed to identify exactly why FtsZ(Ts1) is unable to complete this process at 49°C. During this work, it was discovered that in the presence of elevated levels of the FtsZ-binding protein ZapA, FtsZ(Ts1) regains the capacity to form functional Z rings

at 49°C via the normal assembly pathway. The ZapA protein has previously been shown to promote Z ring assembly in the cell, and to stimulate the association of simple FtsZ polymers (protofilaments) into higher-order polymeric structures *in vitro*. These and other results suggest that FtsZ(Ts1) is specifically defective in its ability to support higher-order polymer association *in vivo* under non-permissive conditions. This enables FtsZ(Ts1) to polymerise into a helix, while preventing the helix from undergoing the structural changes required for it to reorganise into a stable ring. These findings have important implications regarding the molecular mechanism of the FtsZ helix-to-ring transition in wild-type cells.

Other work presented in this thesis involved a genetic screen for extragenic suppressors of *tsI* thermosensitivity. Using insertional mutagenesis, a total of four unique genes were identified that rescue *tsI* to temperature resistance when inactivated. Given that the *tsI* strain is specifically defective in FtsZ function, and taking into account other findings in the literature, this strongly suggests that these genes act in some capacity to control FtsZ activity *in vivo*. Further characterisation of the gene products promises to uncover novel insights into the regulation of Z ring assembly in bacteria.